U.S. DEPARTMENT OF AGRICULTURE GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION FEDERAL GRAIN INSPECTION SERVICE STOP 3630 WASHINGTON, DC 20090-3630 AFLATOXIN HANDBOOK CHAPTER 20 4-10-06

## CHAPTER 20

## QUICKTOX TEST KIT (Released 2006)

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#### 20.1 GENERAL INFORMATION

The QuickTox test kit uses lateral flow test strip technology that provides qualitative (equal to or less than a specified threshold) results.

### 20.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the QuickTox test method is either a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (Reagent grade or better) and 30 percent water or 50 percent ethanol (Reagent grade or better) and 50 percent water.

## a. <u>Methanol/Water</u>

- (1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- (2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

## b. <u>Ethanol/Water</u>

- (1) Using a graduated cylinder, measure 500 ml of ethanol and place it into a clean carboy with spigot.
- (2) Add 500 ml deionized or distilled water to the ethanol and shake vigorously until it is completely mixed.

- (3) Label the container stating the mixture (50 percent ethanol and 50 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 1 part ethanol to 1 part deionized or distilled water.

#### 20.3 EXTRACTION PROCEDURES

- a. Transfer 50 grams of ground sample into an extraction mixing jar.
- b. Add 100 ml of the (70/30) methanol/water or (50/50) ethanol/water extraction solvent.
- c. Cover the extraction jar and shake by hand for 2 minutes. If a mechanical shaker is used shaking time may be reduced to 1 minute.
- d. After shaking, the sample will immediately begin to separate into 2 layers. The top (yellowish) layer containing the aflatoxin residues will be used for testing.

## 20.4 TEST PROCEDURES

- a. Reaction Vial.
  - (1) Using the fixed volume pipette included in the test kit, place 150 microliters (150 µl) **tap** water into a reaction vial.
  - (2) Using the same fixed volume pipette, remove 150 µl from the top (yellowish) layer of the extract. Add the extraction solution to the reaction vial containing water.
  - (3) Mix water and sample extraction solution by stirring with the tip of the fixed volume pipette.

**NOTE:** To ensure correct volumes are used to prepare the test sample, a fixed volume pipette is included with the kit. When a liquid drawn to the top of the straw end of the pipette is dispensed, 150  $\mu$ l will be expelled into the reaction vial. Any overfill is retained in the pipette. After diluting the sample the final volume in the reaction vial should be 150  $\mu$ l. Do not reuse diluted samples. Use a new fixed volume pipette and reaction vial for each sample.

### b. <u>Test Strips.</u>

- (1) Allow refrigerated canisters to come to room temperature before opening. Remove the QuickTox strips to be used then immediately reseal the canister. Avoid bending the strips. Use care not to dislodge the arrow tape on the end of the strip.
- (2) Place the strip into the reaction vial containing the diluted sample extract. The arrow tape on the end of the strip should point into the reaction vial.
- (3) The sample extract will travel up the strip. Reaction vials will stand on their own or may be inserted into the cardboard racks provided.
- (4) Allow the strip to develop for 5 minutes before making final assay interpretations. Negative sample results may become obvious more quickly (2-3 minutes).
- (5) If the strips are to be retained, cut off and discard the bottom section of the strip covered by the arrow tape.

## c. Interpreting the Lateral Flow Test Strip.

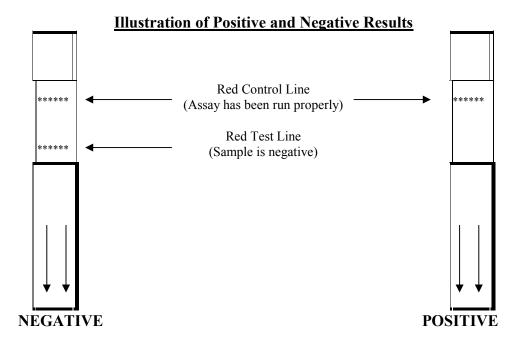
Development of a Control Line within 5 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh 1:2 dilution) should be made and tested using another strip.

#### (1) Negative Result.

A sample containing aflatoxin residues less than or equal to 20 ppb will develop 2 distinct lines, the Control Line and the Test Line, in the test area.

# (2) <u>Positive Result.</u>

A sample containing aflatoxin residues in excess of 20 ppb will develop 1 distinct line, the Control Line.



### 20.5 REPORTING AND CERTIFYING TEST RESULTS

- a. Report results on the pan ticket and inspection log as being equal to or less than 20 ppb ( $\leq$  20 ppb), or as exceeding 20 ppb ( $\geq$  20 ppb), as applicable.
- b. Certify results as being equal to or less than 20 ppb or exceeding 20 ppb, as applicable.
- c. Refer to the Certification section of the handbook for more detailed certification procedures.

#### 20.6 CLEANING LABWARE

### a. Negative Tests ( $\leq 20 \text{ ppb}$ ).

#### (1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, then rinse with clean water before reusing.

## (2) <u>Disposable Materials.</u>

Place materials in a garbage bag for routine trash disposal.

## b. <u>Positive Tests (> 20 ppb).</u>

### (1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

#### (2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes, for at least 5 minutes.

Pour off the liquid down the drain and place the materials in a garbage bag and discard

#### 20.7 WASTE DISPOSAL

a. Negative Results ( $\leq 20 \text{ ppb}$ ).

If the test result is negative (equal to or less than 20 ppb), dispose of any remaining liquid filtrate in the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

b. <u>Positive Results (> 20 ppb).</u>

If the result is positive (more than 20 ppb), the slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, pour approximately 50 ml of bleach solution into the sample extraction jar and shake to mix with the sample slurry.

After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution and dispose of by pouring the liquid down the down. Discard the sample slurry (ground portion) paper into a plastic garbage bag for disposal.

### 20.8 EQUIPMENT AND SUPPLIES

- a. Materials Supplied in Test Kits
  - (1) 50 QuickTox strips packed in a moisture-resistant container.
  - (2) 50 fixed volume transfer pipettes.
  - (3) 50 reaction vials.
- b. <u>Materials Required but not Provided:</u>
  - (1) Timer (5 minute capacity).
  - (2) Felt tipped pens.
  - (3) Balance.
  - (4) Sample Grinder.

- (5) Methanol Reagent grade or better.
- (6) Ethanol Reagent grade or better.
- (7) Deionized or Distilled water.
- (8) Sample extraction jars.
- (9) Orbital/rotary shaker.
- (10) Tap water.

## **20.9 STORAGE CONDITIONS**

a. <u>Storage Conditions.</u>

Test kits should be refrigerated between 36°- 48°F.

- b. Precautions.
  - (1) Do not use the test kits beyond the noted expiration date.
  - (2) Prolonged exposure to high temperatures may adversely affect the test results.
  - (3) Do not open the desiccated canister until ready to use the strips.